A Flow Chart for Array-based Detection of Gene Expression

Hybridization Oligo:

| | D | EX | Zip | U | |

U: Upstream universal priming site
Zip: Unique sequence as a molecular "zip-code"
EX: Gene-specific exon sequence
D: Downstream universal priming site

Exon Sequence

↓

mRNA: Cap ————————————————— $(A)_n$

Hybridize to Total RNA
or Poly(A) + mRNA:

↓

Exon Sequence

↓

Cap ————————————————— $(A)_n$

3'   5'

Amplify Signal by PCR:
(D Primer is Biotinylated)

↓

B ——→   ←——
3' ▨▨▨| |✕✕✕✕✕✕|▨▨▨▨ 5'

Hybridize and Detect:

↓

S ◁ B ——●——✕✕✕✕✕✕✕✕——— 3'
            ✕✕✕✕✕✕✕✕
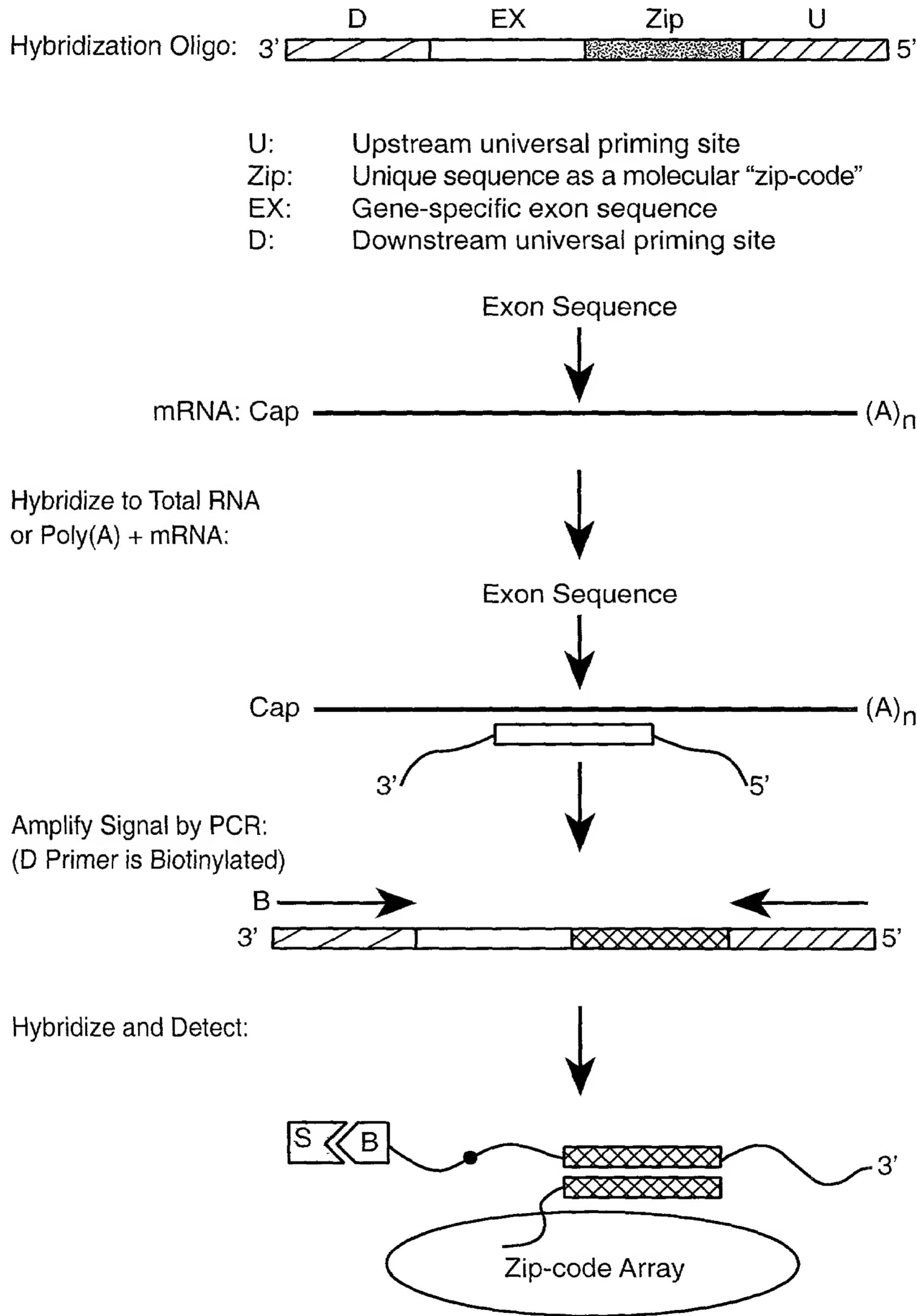
Zip-code Array

FIG._1

A Flow Chart for Array-based Detection of RNA Alternative Splicing

Hybridization Oligo: 3' [D | SJ | Zip | U] 5'

U:	Upstream universal priming site
Zip:	Unique sequence as a molecular "zip-code"
SJ:	Gene-specific splice junction
D:	Downstream universal priming site

Splice Junction

mRNA: Cap ——————————————————— (A)$_n$

Hybridize to Total RNA
or Poly(A) + mRNA:

Splice Junction

Cap ——————————————————— (A)$_n$

Amplify Signal by PCR:
(D Primer is Biotinylated)

B ——→

3' [| | |] 5'

Hybridize and Detect:

[S ⟨ B]

Zip-code Array
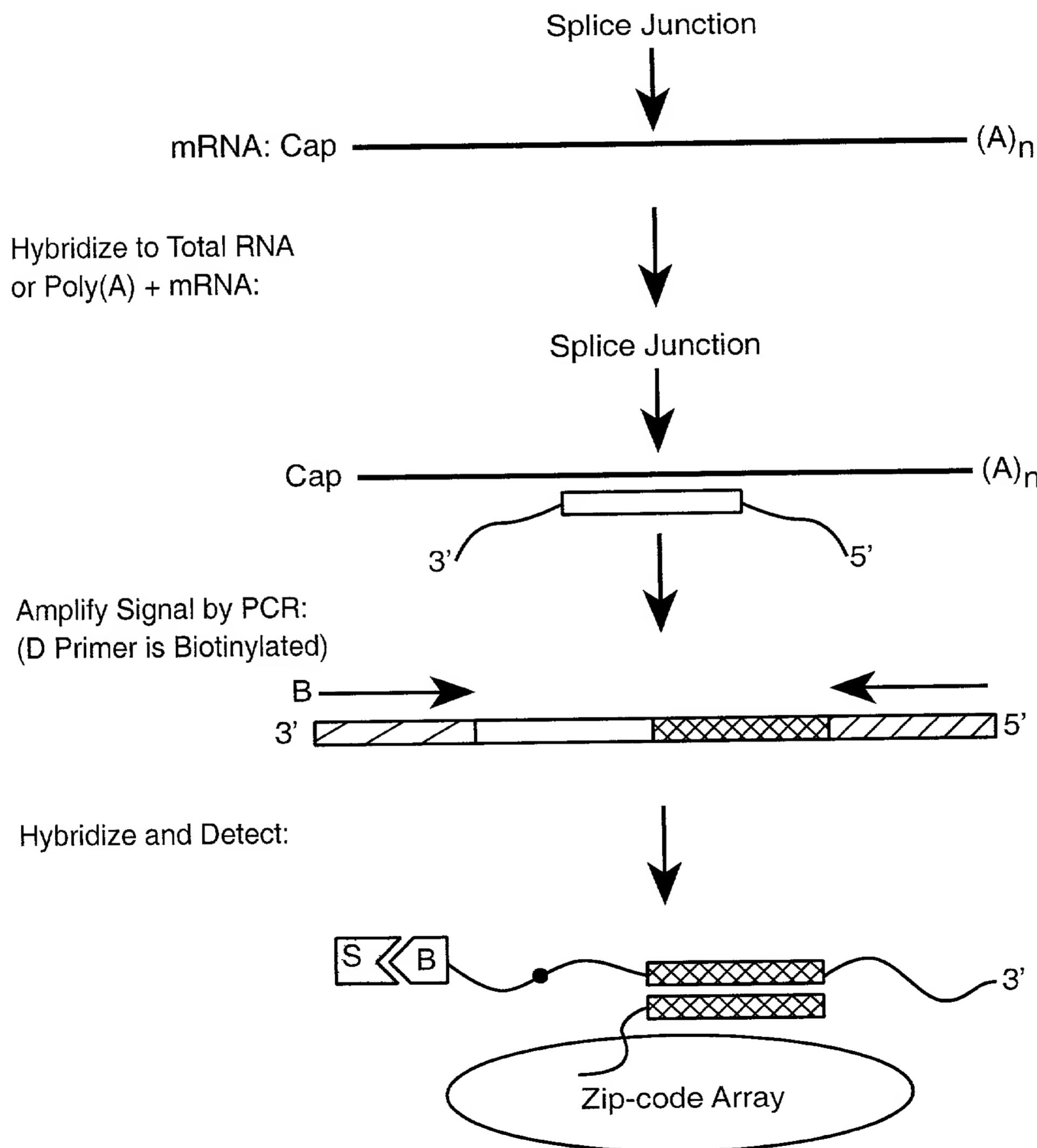
FIG._2

Genome-wide Gene Expression Profiling Using Oligo-ligation Strategy

Upstream Oligo: 3' [EX1 | Zip 1 | U] 5'

Downstream Oligo: 3' [D | Zip 2 | EX2] 5'

U:	Upstream universal priming site
Zip 1:	Unique sequence as a molecular "zip-code"
Zip 2:	A different zip-code
EX1:	Gene-specific exon sequence
EX2:	Gene-specific exon sequence
D:	Downstream universal priming site

Exon Sequence

mRNA: Cap ———————————————— (A)n

Hybridize to Total RNA
or Poly(A) + mRNA:

mRNA: Cap ———————————————— (A)n

Ligate the Oligos:

3'   5'

mRNA: Cap ———————————————— (A)n

Amplify Signal by PCR:
(D Primer is Biotinylated)

3'   5'

B ——————→   ←——————

3' [| | | | | | | | |] 5'

Hybridize to the Zip-code Array and
Stain with Labeled Streptavidin:

[S < B] —— [///] —— •—— [XXX] —— 3'
                          [XXX]
                   Zip 1

Zip-code Array

[S < B] —— [///] —— •—— [XXX] —— 3'
           [///]
           Zip 2
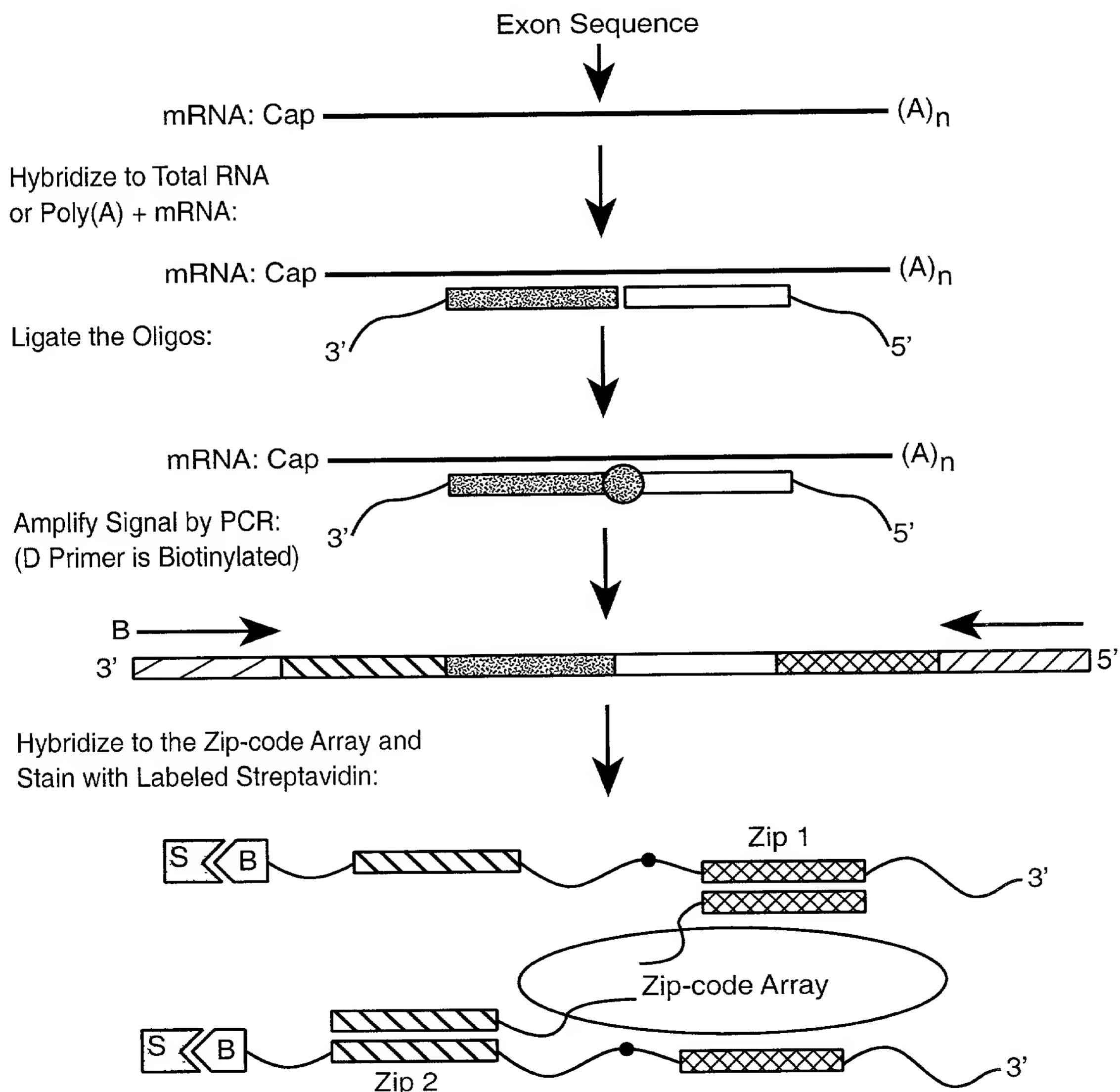
FIG._3

Genome-wide RNA Alternative Splicing Monitoring Using Oligo-Ligation Strategy

Upstream Oligo:

Downstream Oligo:

U: Upstream universal priming site
Zip 1: Unique sequence as a molecular "zip-code"
Zip 2: A different zip-code
SJ: Gene-specific splice junction
ES: Exonic sequence adjacent to the splice junction
D: Downstream universal priming site

Splice Junction

mRNA: Cap ————————————●———————————— $(A)_n$

Hybridize to Total RNA   Splice Junction
or Poly(A) + mRNA:

mRNA: Cap ————————————●———————————— $(A)_n$

Ligate the Oligos:

Splice Junction

mRNA: Cap ————————————●———————————— $(A)_n$

Amplify Signal by PCR:
(D Primer is Biotinylated)

Hybridize to the Zip-code Array and
Stain with Labeled Streptavidin:

Zip 1

Zip-code Array

Zip 2

FIG._4

Direct Genotyping Using a Whole-genome Oligo-ligation Strategy

Upstream Oligo: 3' SNP_A Zip 1 U 5'

Downstream Oligo: 3' D Zip 2 SNP_B 5'

U: Upstream universal priming site
Zip 1:   Unique sequence as a molecular "zip-code"
Zip 2:   A different zip-code
SNP_A: SNP-specific sequence
SNP_B: SNP-specific sequence
D: Downstream universal priming site

Polymorphic Site

Genomic DNA:

Hybridize to Genomic DNA:

Genomic DNA:

Ligate the Oligos: 3'   5'

Genomic DNA:

Amplify Signal by PCR: 3'   5'
(D Primer is Biotinylated)

B   ←
3'   5'

Hybridize to the Zip-code Array and
Stain with Labeled Streptavidin:

Zip 1

S B   Zip 1   3'

Zip-code Array

S B   3'
        Zip 2

FIG._5

Whole-genome Oligo-ligation Strategy

Upstream Oligo:
Target_U   Zip 1   U
3'   5'

Downstream Oligo:
D   Zip 2   Target_D
3'   5'

Middle Oligo:
Target_M
3'   5'

U: Upstream universal priming site
Zip 1:   Unique sequence as a molecular "zip-code"
Zip 2:   A different zip-code
Target_U: Upstream target-specific sequence
Target_D: Downstream target-specific sequence
Target_M: Middle target-specific sequence
D: Downstream universal priming site

Target:

Hybridize to Target:

Target:

Ligate the Oligos:
3'   5'

Target:
3'   5'

Amplify Signal by PCR:
(D Primer is Biotinylated)

B →
3'   5'

Hybridize to the Zip-code Array and
Stain with Labeled Streptavidin:

S B   Zip 1   3'

Zip-code Array

S B   3'
Zip 2

FIG._6

Unhybridize
Probes

Removal / Wash
Of Unhybridized
Probes

Denature

Amplify

FIG._7

FIG._8

FIG._9

Ligation,
Denaturation

Addition
Of Primer,
Extension

FIG._10

Alternative Splicing Targets Selected for Microarray Analysis

1. GAPDH (constitutive splicing control, signal normalization).

2. FGFR2 / KGF (mutually exclusive exons, internal cell type control):

KGFR (epithelial cells)

FGFR2 (fibroblasts)

3. Bcl-x (alternative 5' ss):

Bcl-xL (inhibits PCD)

Bcl-xS (enhances PCD)
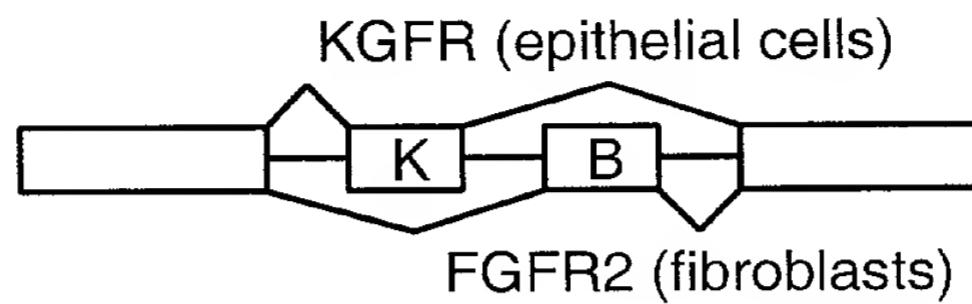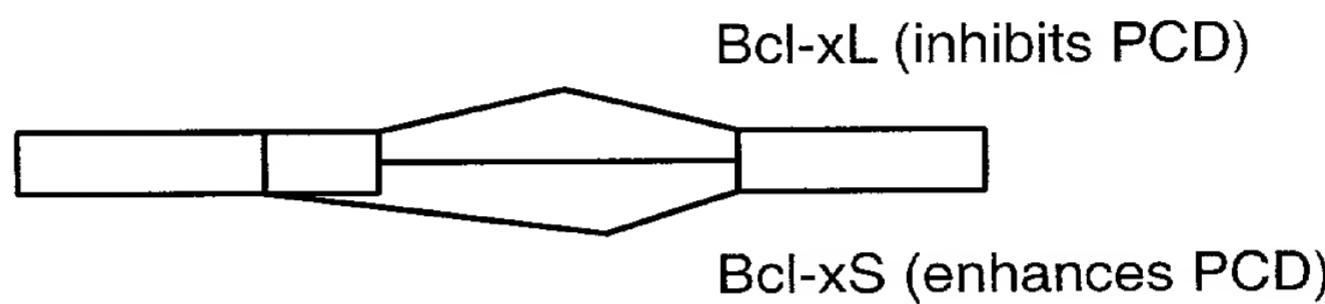
4. c-src (exon inclusion / exclusion):

N-src

C-src
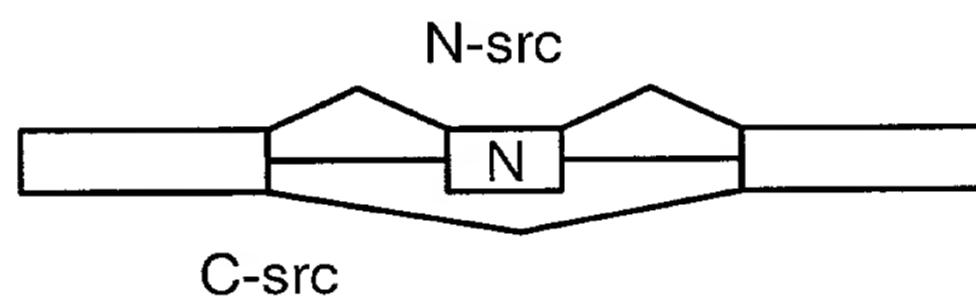
5. CASP2 (exon inclusion / exclusion):

Ich-IS (inhibits PCD)

Ich-IL (enhances PCD)

6. CASP9 (alternative 5' ss):

9

9b (dominant negative form)
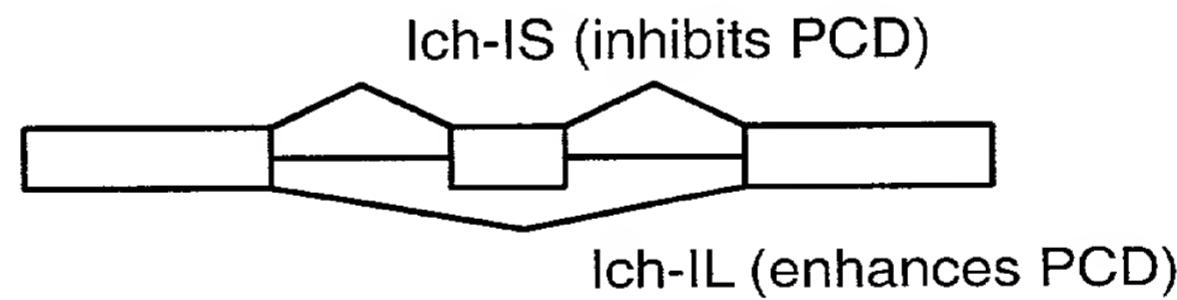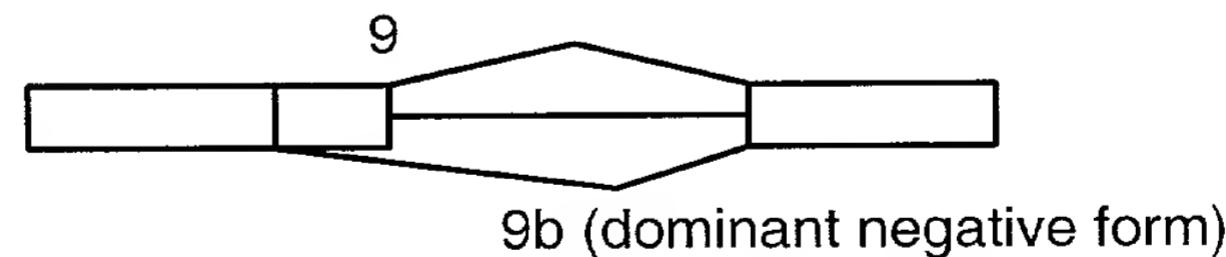
7. Fyn (src family tyrosine kinase, mutually exclusive exons);

brain-specific

thymus specific
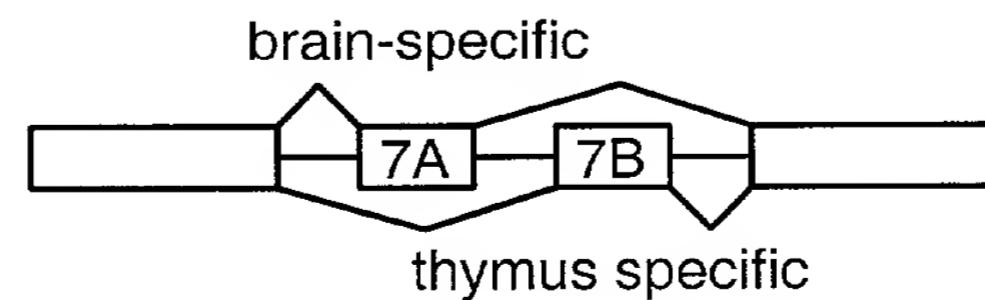
8. NOS1 (alternative promoters / alternative 5' ss):

FIG._11